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Ruhanen, Hanna

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Angiopoietin-like protein 3, an emerging cardiometabolic therapy target with systemic and cell-autonomous functions

Hanna Ruhanen[1,2], P.A. Nidhina Haridas[1], Matti Jauhiainen[1], and Vesa. M. Olkkonen[1,3*]

[1]Minerva Foundation Institute for Medical Research, Helsinki, Finland

[2]Molecular and Integrative Biosciences, University of Helsinki, Finland

[3]Department of Anatomy, Faculty of Medicine, University of Helsinki, Finland

*Corresponding author at: Minerva Foundation Institute for Medical Research, Biomedicum 2U, Tukholmankatu 8, FI-00290 Helsinki, Finland; Tel +358-50-4112297, e-mail vesa.olkkonen@helsinki.fi

ABSTRACT

Angiopoietin like protein 3 (ANGPTL3) is best known for its function as an inhibitor of lipoprotein and endothelial lipases. Due to the capacity of genetic or pharmacologic ANGPTL3 suppression to markedly reduce circulating lipoproteins, and the documented cardioprotection upon such suppression, ANGPTL3 has become an emerging therapy target for which both antibody and antisense oligonucleotide (ASO) therapeutics are being clinically tested. While the antibody is relatively selective for circulating ANGPTL3, the ASO also depletes the intra-hepatocellular protein, and there is emerging evidence for cell-autonomous functions of ANGPTL3 in the liver. These include regulation of hepatocyte glucose and fatty acid uptake, insulin sensitivity, LDL/VLDL remnant uptake, VLDL assembly/secretion, polyunsaturated fatty acid (PUFA) and PUFA-derived lipid mediator content, and gene expression. In this review we elaborate on (i) why ANGPTL3 is considered one of the most promising new cardiometabolic therapy targets, and (ii) the present evidences for its intra-hepatocellular or cell-autonomous functions.

Keywords: Angiopoietin-like 3, lipoprotein lipase, lipid metabolism, intracellular function, hepatic lipids, therapy target

1. Introduction

Angiopoietin like 3 (ANGPTL3) belongs to the angiopoietin-like protein family consisting of 8 members structurally similar to angiopoietins [1]. ANGPTL3 was first identified by Conklin et al. [2]. It is mainly expressed in liver; very low expression is also detectable in kidney podocytes but its role in podocyte function is not fully understood. The protein consists of an N-terminal signal peptide, a coiled-coil region, a linker region, and a C-terminal fibrinogen-like domain (FLD; Fig. 1). ANGPTL3 expression is regulated positively by liver X receptor (LXR) and negatively by insulin, leptin and thyroid hormone [3-6]. The main function of ANGPTL3 is in the regulation of lipid metabolism, where it acts synergistically with the related ANGPTL4 and ANGPTL8, forming a functionally essential protein-protein complex with the latter [7,8]. Unlike ANGPTL3, ANGPTL4 and ANGPTL8 are expressed in both adipose tissue and liver [6,9,10].

Angptl3 was in an early study found to associate with a hypolipidemia trait in a moderately obese mouse strain [11], and knock-out of *Angptl3* was reported to result in a hypotriglyceridemia phenotype [12]. Later, genome-wide association study (GWAS) in human subjects revealed that markers in the region of *ANGPTL3* are associated with circulating triglyceride (TG) levels [13]. Moreover, loss-of-function (LOF) variants of *ANGPTL3* were found to associate with reduced plasma LDL- and HDL-cholesterol (LDL-C, HDL-C) and TG [14]; Several other studies soon replicated these observations [10,15,16]. The major known function of ANGPTL3 is to inhibit the activity of lipoprotein lipase (LPL), an enzyme that hydrolyses TG in TG-rich lipoproteins (TRL) [10,17]. Moreover, ANGPTL3 also inhibits endothelial lipase (EL) [18], which provided a putative explanation for its effects on circulating HDL-C as shown *in vivo* in mouse [19]. However, ANGPTL3 deficient human subjects displayed similar EL activity as in control individuals, suggesting that other mechanisms may account for the reduction of HDL levels in these subjects [16]. Importantly, *ANGPTL3* LOF variant carriers are protected from coronary artery disease [20]. Due to this cardioprotective effect, ANGPTL3 has emerged as a novel therapeutic target for cardiovascular diseases (CVD). ASO and monoclonal antibody-based therapeutics targeting ANGPTL3 are in clinical trials [21-25]. While the function of ANGPTL3 in regulating plasma lipoprotein levels is rather well studied and reviewed [1,26-29], very little is known about the putative intracellular functions of ANGPTL3. Since *ANGPTL3*-targeting ASO drugs, which inhibit the hepatocellular expression of the protein, are under clinical trial, it is important to also understand the intracellular functions of ANGPTL3. In this review we

address two crucial aspects of the protein: (i) Why is ANGPTL3 considered a promising therapeutic target for CVD? (ii) What is the evidence for intra-hepatocellular or cell-autonomous functions of ANGPTL3, and what are the implications of such functions?

2. Why is ANGPTL3 a promising cardiovascular therapy target?

Due to its role as a crucial physiologic regulator of LPL and EL activity, ANGPTL3 has a major impact on the circulating lipoproteins, the loss of its function resulting in marked reductions of all major lipoprotein classes. Moreover, evidence from pre-clinical studies suggests that inactivation of ANGPTL3 reduces VLDL-TG secretion [19,23,30,31] and enhances the hepatic uptake of apoB100-containing lipoproteins [32] as well as VLDL-TG uptake into oxidative tissues [33]. The role of ANGPTL3 in human lipid metabolism was revealed in 2010 through the identification of individuals carrying LOF mutations in *ANGPTL3* [14]. Genetic deficiency of *ANGPTL3* was found to cause familial combined hypolipidemia (FHBL2, OMIM #605019) characterized by very low plasma TG, LDL-C and HDL-C concentrations. A number of LOF mutations have been characterized in the gene [14,34-36]. Of these, *ANGPTL3* S17X is particularly interesting since it completely eliminates the protein and has been detected in three generations in the town of Campodimele in Italy [36,37]. The life expectancy of both females and males in this small town is exceptionally high, approximately 95 y. The inbred inheritance of *ANGPTL3* LOF mutations is considered one of the factors underlying this remarkable longevity.

A crucial question has arisen whether genetic abrogation of *ANGPTL3* associated with reduced circulating lipoprotein concentrations is protective against CVD. An extensive meta-analysis (a total of 21,980 CVD patients and 158,200 controls) demonstrated that carriers of *ANGPTL3* LOF mutations have 34% lower odds of CVD than control subjects [20]. Individuals belonging to the lowest tertile of circulating ANGPTL3 concentration presented with a 29% lower myocardial infarction (MI) risk than the highest tertile. A reduction of CVD risk among *ANGPTL3* LOF mutation carriers was replicated by Dewey et al. [21], who sequenced *ANGPTL3* exons in 58,335 participants of the DiscovEHR genetics study. Here, subjects heterozygous for *ANGPTL3* LOF variants displayed significantly lower serum TG (–27%), HDL-C (–4%) and LDL-C (–9%) concentrations than participants lacking the variants. Moreover, analysis of *ANGPTL3* LOF variants in 13,102 coronary artery disease (CAD) patients and 40,430 controls showed that the LOF variants

were found in 0.33% of the cases and in 0.45% of the controls (adjusted odds ratio, 0.59; 95% CI, 0.41 to 0.85; p=0.004). These intriguing observations were further confirmed by follow-up studies employing four large population cohorts. An inverse-variance-weighted fixed-effects meta-analysis combining the DiscovEHR and the other population studies suggested an odds ratio of 0.61 (95% CI, 0.45 to 0.81; p<0.001) [21]. Interestingly, Lotta et al. [38] reported in a large human material (392,220 participants) that *ANGPTL3* LOF variants were associated with greater protection against coronary disease than other LDL-C-lowering genetic mechanisms (*ANGPTL3* loss-of-function variants: odds ratio, 0.66; 95% CI, 0.52-0.83; 58 other LDL-C-lowering variants: odds ratio, 0.90; 95% CI, 0.89-0.91; P for heterogeneity = 0.009).

The above epidemiological studies suggested that the plasma lipid/lipoprotein lowering observed in *ANGPTL3* LOF carriers (the strongest effect being detected on TG levels) may precipitate as a drop of cardiovascular risk. The anticipated adverse effects of HDL lowering were apparently overridden by the beneficial reduction of TG and LDL-C. Reduction or inactivation of ANGPTL3 was, based on the above observations, considered a promising candidate CVD therapeutic strategy, and is being actively pursued.

What have we learned from the clinical trials on ANGPTL3 targeting? Trials on the suppression or inhibition of ANGPTL3 are ongoing at phases 2 and 3. The therapeutic agents under study are antisense oligonucleotides (AKCEA/IONIS-ANGPTL3-L_{Rx}, a GalNac-conjugated ASO, presently called Vupanorsen), which suppress the hepatic expression of *ANGPTL3*, or a human monoclonal antibody that inactivates circulating but not intra-hepatic ANGPTL3 (Evinacumab, Regeneron Pharmaceuticals). Moreover, *in vivo* genome editing by CRISPR-Cas9 technology is being developed as a tool to introduce LOF mutations in *ANGPTL3.* This novel approach has yielded the first promising results in a mouse model [39]. Results from both the ASO and the antibody trials have revealed significant decreases of plasma ANGPTL3 levels coinciding with reductions of all major lipoprotein classes. Graham et al. [23] showed in a phase 1 clinical trial that, after 6 weeks of ASO treatment on healthy adults, subjects receiving single or multiple doses of the ASO displayed 46.6 to 84.5% reductions of plasma ANGPTL3 protein (P<0.01 for all doses vs. placebo) and marked drops in the levels of TG (reductions of 33.2 to 63.1%), LDL-C (1.3 to 32.9%), VLDL-C (27.9 to 60.0%), non-HDL-C (10.0 to 36.6%), apoB-100 (3.4 to 25.7%), and apoC-III (18.9 to 58.8%). Of note, although thrombocytopenia has been reported as an

adverse side effect of certain ASO therapies [40,41], this or other serious treatment-emergent adverse events (TEAE) or discontinuation of the therapy were not documented during the trial.

After successful lipid lowering in mice and cynomolgus monkeys [19,21], the monoclonal antibody Evinacumab was administered in 83 healthy human volunteers with mildly to moderately elevated TG (150-450 mg/dl) or LDL-C (100 mg/dl) by Dewey et al. [21]. This resulted in a dose-dependent reduction in fasting TG of up to 76% and LDL-C of up to 23%. Similar observations were recently reported from a clinical trial on hypertriglyceridemic subjects by Ahmad et al. [24], who also measured significant reductions of VLDL-C in the Evinacumab-treated groups, in the absence of serious TEAE or discontinuation of the therapy.

When one considers subjects with familial hypercholesterolemia (FH), for whom the efficacy of statin and PCSK9 inhibitor therapies is often not sufficient to reach their target lipid levels, ANGPTL3 targeting might give additional benefit. Consistent with this idea, Evinacumab administered for 4 weeks on top of intense lipid-lowering therapy resulted in a further reduction of TG (by 47%), LDL-C (by 50%), and HDL-C (by 36%) in a group of 9 homozygous FH patients [22]. A more extensive phase 3 trial (ELIPSE HoFH; NCT03399786) on homozygous FH patients is ongoing with promising 1st endpoint results (49% reduction of LDL-C; significant drop of TG, TC, non-HDL-C and apoB). The idea of combining Evinacumab with other lipid-lowering therapies to reach maximal efficiency was recently tested in a pre-clinical setting by Pouwer et al. [42]. The authors studied the effect of ‘triple therapy’ consisting of evinacumab, the PCSK9 inhibitor alirocumab, and atorvastatin, on atherosclerosis in APOE*3-Leiden.CETP mice, a well-established model mimicking human hyperlipidemia. After 13-week western-type diet the mice received the pharmaceuticals for 25 weeks, the ‘triple therapy’ yielding clearly better results than atorvastatin alone or atorvastatin + alirocumab (‘double treatment’). Atorvastatin alone reduced lesion progression by 28%, the double treatment completely blocked lesion progression and diminished lesion severity, increasing their collagen content, while the triple therapy regressed the lesions in thoracic aorta by 50% and in the aortic root by 36%, increasing their collagen and reducing their macrophage content. All the above pre-clinical and clinical trials strongly suggest that residual risk remaining after statin-PCSK9 inhibitor treatment can be further diminished via blocking ANGPTL3 function. It is noteworthy that both the ASO and

antibody therapies elicited apoB reductions ranging from -14% to -46%. Considering a recent report suggesting that the clinical benefit of TG and LDL-C lowering may in fact be proportional to the absolute change in apoB [43], this may be a crucial effect of ANGPTL3-targeting therapy. The above studies and the evidence in favor of employing ANGPTL3 as cardiometabolic therapy target are summarized in Table 1.

Table 1. Evidence for lipid lowering and cardioprotection upon loss or suppression of ANGPTL3 activity/expression.

Method	Species	Documented effect	Reference(s)
Genetic loss-of-function (LOF)	Human	Reduction of TG, LDL-C, HDL-C	[14]
	Human	Reduction of TG, TC, LDL-C, HDL-C, ApoB, ApoA-I	[15,44]
	Human	LOF carriers, OR[1] of CAD 0.66; Lowest tertile of ANGPTL3 conc. OR 0.65 relat. to highest tertile; Reduction of TG, TC, LDL-C HDL-C	[20]
	Human	LOF carriers, OR of CAD 0.59; Reduction of TG, TC[2], LDL-C, HDL-C	[21]
	Human	LOF carriers, OR of CHD 0.66; Other LDL-C lowering variants, 0R 0.90	[38]
	Human	Reduction of TG, TC, LDL-C, HDL-C, ApoB, ApoA-I, FFA; Increase of IS[3]	[16]
	Mouse, *Angptl3*$^{-/-}$	Reduction of TG, TC; Increase of IS and WAT[4] glucose uptake	[12,33]
Antibody inhibition	Human	Reduction of TG (max –76%)[5], LDL-C (–23%), HDL-C (–18%)	[21] NCT01749878
	Human (FH[6])	Reduction of LDL-C (mean –49%), apoB (–46%), non-HDL-C (–49%), TG (–47%), HDL-C (–36%)	[22] NCT02265952
	Human (FH)	Reduction of LDL-C (–49%), TG, TC, non-HDL-C and apoB	NCT03399786
	Human (hyper-TG)	Reduction of TG (max –88%), TC (–34%), LDL-C (–25%), non-HDL-C (–40%), VLDL-C (–91%), apoB (–31%), HDL-C (–28%)	[24] NCT01749878 NCT02107872
	Cynomolgus monkey	Reduction of TG, non-HDL-C, HDL-C	[19]
	Mouse, *APOE*3Leiden.CETP*[7]	Lesion area reduction 39% Necrotic area reduction 45% Reduction of TG, TC. VLDL-C	[21]
	Mouse, WT, *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$, *Lrp1*$^{-/-}$, *Sdc1*$^{-/-}$	Reduction of TG, TC, LDL-C, VLDL-C	[31]
	Mouse, WT, dyslipidemic	Reduction of TG, TC, LDL-C, HDL-C	[19]

	Mouse, *APOE*3Leiden.CETP*	Evinacumab + alirocumab + atorvastatin, lesion regression; Reduction of TC, non-HDL-C, VLDL-C, LDL-C	[42]
ASO	Human	Reduction of TG (max –63%), LDL-C (–33%), VLDL-C (–60%), non-HDL-C (–37%), ApoB (–26%), ApoC-III (–59%), ApoA-I, Lp(a)	[23] NCT02709850
	Mouse, *Ldlr*$^{-/-}$	Lesion area reduction 52% Reduction of TG, LDL-C	[23]
siRNA	Mouse, WT, *ob/ob hCETP/ApoB-100 Apobec*$^{-/-}$ *hApoB Tg Ldlr*$^{-/-}$	Reduction of TG, HDL-C, LDL-C Reduction of of TG, LDL-C Minimal effect on LDL-C	[32]
CRISPR-Cas9 base editing	WT, *Ldlr*$^{-/-}$	Reduction of TG, TC	[39]

[1]Odds ratio; [2]Total cholesterol; [3]Insulin sensitivity; [4]White adipose tissue, [5]For human clinical studies a maximum or mean (for ref. [22]) % reduction of plasma lipids or lipoproteins are given. The numbers are not directly comparable since they have been picked from different single or multiple dose regimes in the different studies. [6]Familial hypercholesterolemia; [7]Cholesterol ester transfer protein

To conclude, ANGPTL3 is currently considered a highly promising cardiovascular therapy target. Its inactivation alone or especially in combination with other lipid-lowering therapies holds great promise particularly for subjects with hard-to-treat dyslipidemias inflicting a high CVD risk. Moreover, additional benefit concerning metabolic disease is suggested by the observations demonstrating that ANGPTL3 deficiency is associated with increased insulin sensitivity in both human subjects [16] and mice [33]. Even though the Evinacumab antibody therapy has shown positive results, the ASO therapy and the emerging CRISPR-Cas9 approach are likely to gain ground in the future. However, both of these induce long-term or permanent suppression of the hepatic *ANGPTL3* expression, thus also afflicting the putative intra-hepatocellular or cell-autonomous functions of ANGPTL3 – detailed study of these functions is therefore a necessity.

3. ANGPTL3 and non-alcoholic fatty liver disease (NAFLD)

3.1 Familial hypobetalipoproteinemias and fatty liver

As indicated above, LOF mutations in the *ANGPTL3* gene cause familial combined hypolipidemia 2 (FHBL2, OMIM #605019) associated with significantly reduced plasma levels of all apoB-100 containing lipoproteins as well as HDL. Also several variants of the

APOB gene are connected to low plasma apoB-100 and LDL-C concentrations, causing familial hypobetalipoproteinemia 1 (FHBL1, OMIM #615558). In FHBL1 apoB-100 plasma levels are generally <5th percentile whereas LDL-C concentrations are in the range of 200-500 mg/L [45,46]. Of note, FHBL1 subjects are prone to develop NAFLD [47-49]. Using stable isotope techniques it was demonstrated that heterozygous FHBL1 subjects have a significantly reduced hepatic apoB-100 secretion rate, attenuated production of LDL-associated apoB-100, and highly enhanced catabolism of VLDL particles [50,51].

Although an elevated prevalence of liver steatosis in FHBL1 has been firmly documented, the hepatic neutral lipid levels in FHBL2 are as yet not well established. There are only a few investigations addressing the hepatic lipid status in *ANGPTL3* deficient subjects. Musunuru et al. [14] characterized one *ANGPTL3* LOF mutation kindred, and signs of fatty liver were not detected, suggesting that ultrasonography could be employed to discriminate *ANGPTL3* (FHBL2) from *APOB* deficiency (FHBL1), but not from hypobetalipoproteinemia due to *PCSK9* mutations, where advanced fatty liver is usually not observed. Minicocci et al. [44] studied 115 FHBL2 individuals with 13 different *ANGPTL3* variants (14 homozygotes, 8 compound heterozygotes, and 93 heterozygotes) and about 400 control subjects. Detailed examination for the presence of hepatic steatosis was performed among 64 *ANGPTL3* S17X variant carriers and 103 control subjects. The authors did not observe increased prevalence of liver steatosis in this FHBL2 group compared with the non-carrier controls. Furthermore, there was no association between the severity of steatosis and the FHBL2 status. Di Costanzo et al. [52] carried out a direct comparison of the hepatic lipid phenotypes of FHBL1 and FHBL2. Altogether 350 subjects, 67 heterozygous carriers of *APOB* mutations (FHBL1 subjects), 57 heterozygote and 6 homozygote ANGPTL3 variant carriers (FHBL2 subjects), and 220 normolipidemic controls were included. Prevalence and degree of hepatic steatosis were analyzed by ultrasonography. The major outcome of this study was that, when comparing to the controls, the prevalence and severity of hepatic steatosis were significantly increased in heterozygous FHBL1 subjects ($P<0.001$), while the FHBL2 participants did not differ from controls. The hepatic consequences of FHBL2 thus seem to be strikingly different from those of *APOB*-linked FHBL1 in which elevated prevalence of hepatic steatosis has been consistently demonstrated.

3.2 Plasma ANGPTL3 concentration, insulin resistance and liver disease

Experimental studies have suggested a potential role of the angiopoietin-like proteins, especially ANGPTL3, in the pathogenesis of metabolic syndrome, hepatic steatosis, and more advanced forms of liver disease. One study focused on measuring ANGPTL3 plasma levels in patients with definite nonalcoholic steatohepatitis (NASH, n=40), borderline NASH (n=8), simple fatty liver (n=9), and healthy control subjects without liver disease (n=14). The results suggested that plasma ANGPTL3 levels are increased in the more severe cases of NAFLD in association with insulin resistance [53]. Another single center, cross-sectional study among approximately 1000 Japanese undergoing routine health checks also observed elevated circulating ANGPTL3 concentrations in subjects with impaired liver function and hepatic inflammation [54]. The DiOGenes (Diet, Obesity and Genes) study examined the role of ANGPTL3 in lipoprotein metabolism and liver health status [55]. The authors measured ANGPTL3 levels in relation to body mass index, plasma lipid profile, and markers of hepatic steatosis before and during weight loss. Moreover, genetic variants affecting plasma ANGPTL3 levels were defined through protein quantitative trait locus (pQTL) analysis. The major findings were a strong negative correlation between plasma ANGPTL3 concentration and ASAT activity, and a positive association with cytokeratin 18 (CK-18), independent of weight loss. CK-18 is a major hepatic intermediate filament protein, and plasma CK-18 acts as a biomarker for the apoptotic death of hepatocytes, NASH and hepatic inflammation [56]. The authors of the DiOGenes study suggested that elevation in plasma ANGPTL3 concentration results from hepatic inflammation, or that ANGPTL3 itself could play a role in the development of liver malfunction. *In vitro* support for these suggestions is provided in the study by Szalowska et al. [57], where inflammation was induced *in vitro* in human liver tissue, and ANGPTL3 appeared as a biomarker associated with the inflamed status. Of note, no increase of liver fat content was reported in mice treated with ANGPTL3 ASO, and no significant change in biomarkers of liver function was observed in human subjects who received the ASO [23], in contrast to the hepatotoxicity frequently observed upon treatments with inhibitors targeting the microsomal triglyceride transfer protein (Lomitapide; [58]) or apoB-100 (Mipomersen; [59]).

Interestingly, Ruhanen et al. [60] recently demonstrated that in human hepatocytes subjected to ANGPTL3 knock-down (KD) several lipid classes were enriched in n-6 and n-3 polyunsaturated fatty acids (PUFA), and this phenomenon coincided with an elevation of a number of PUFA-derived lipid mediators relevant for the resolution of inflammation and protection from hepatic insulin resistance and steatosis (see paragraph

7). However, because of the controversy on the applicability of non-invasive biomarkers as metrics of liver derangements, future investigations should analyze liver biopsies to more reliably evaluate the role of ANGPTL3 in liver disease.

4. Modulation of hepatic lipoprotein uptake by ANGPTL3

The reduction of circulating lipoproteins upon *ANGPTL3* LOF can be partially explained by the increase in LPL and EL activity caused by the loss of secreted ANGPTL3. However, the loss of ANGPTL3 also may contribute to this by enhancing hepatic lipoprotein uptake. The decrease in LDL-C upon ANGPTL3 KD *in vivo* in mouse appeared to be mediated via the LDL receptor (LDLR), as suggested by experiments employing Ldlr-deficient *Apobec1*$^{-/-}$*/ApoB-100* transgenic mice [32]. However, contradicting *in vivo* results were reported by Graham et al. [23] who found a similar drop of LDL-C upon *ANGPTL3* ASO-treatment in both wild-type and *Ldlr*$^{-/-}$ mice, implying that the drop of LDL in this case may not depend on functional LDLR. While the best characterized high affinity receptor for LDL is LDLR, a recent LDL transcytosis study by Armstrong et al. [61] showed that LDL co-localized partially with the scavenger receptor SR-BI and overexpression of SR-BI increased LDL transcytosis, whereas SR-BI knockdown by siRNA significantly inhibited this process. This suggests that hepatic SR-BI could facilitate LDL uptake in *Ldlr*$^{-/-}$ mice and thus contribute to the reduced plasma LDL-C levels upon suppression of ANGPTL3, an issue that warrants further study.

Interestingly, deficiency of ANGPTL3 in the HuH7 and HepG2 hepatocellular carcinoma cells *in vitro* was shown to result in an elevated rate of LDL and VLDL uptake. Further, the ANGPTL3 depletion induced intracellular accumulation of long-chain TG and apoB-100, as well as elevated expression of both LDLR and LDLR-related protein 1 (LRP1) [32] (Fig. 2). Since ANGPTL3 function through inhibition of LPL is hardly relevant in experiments employing cultured hepatocytes, the above *in vitro* results suggest that ANGPTL3 modulates LDL uptake through an intracellular, or cell-autonomous mode of action. The study of Wang et al. [31] employing antibody inhibition of ANGPTL3 in mouse did not observe LDLR-dependency of the drop of circulating LDL, and this is no doubt the case also in homozygous human FH patients carrying *LDLR* null mutations but still exhibiting a marked drop of LDL-C upon Evinacumab therapy [22]. These observations suggest that inhibition of the circulating ANGPTL3 by the monoclonal antibody, which most likely dampens the hepatic

VLDL secretion through reduction of FA flux towards the liver, results in a marked drop of circulating LDL independent of the LDLR pathway (Fig. 2; see below).

5. ANGPTL3 regulates hepatocyte VLDL secretion.

Plasma VLDL and LDL levels are very low in ANGPTL3 deficient humans and mice. To what extent can this be attributed to inhibition of VLDL secretion by hepatocytes? Targeting ANGPTL3 with a monoclonal antibody (Evinacumab, REGN1500) in a number of genetically modified mouse models reduced the plasma total levels of TG and cholesterol, including those in the VLDL fraction, and the hepatic TG secretion, without effect on plasma apoB concentration or VLDL/LDL particle numbers [19,21,31]. The study of Wang et al. [31] suggested a selective effect of ANGPTL3 inhibition on hepatic TG but not apoB secretion. Such an effect could be explained by enhanced LPL activity in oxidative tissues and possibly reduced adipose tissue lipolysis [62], resulting in a reduced flux of FA to the liver and, as a consequence, reduced assembly and secretion of large VLDL particles (Fig. 2). A somewhat contradicting observation was made by Xu et al. [32], who reported reduced apoB-100 accumulation in the growth medium of HuH7 and HepG2 cells subjected to ANGPTL3 KD. This finding may also suggest that the consequences of *in vivo* inhibition of circulating ANGPTL3 may include cross-talk between liver and tissues such as WAT, a connection that is lacking when studying hepatocytes in culture. According to Wang et al. [31], the turnover of LDL or VLDL was not affected in mice treated with ANGPTL3-inhibiting antibody, consistent with the notion that the observed β-lipoprotein reduction was largely due to inhibition of VLDL secretion.

ASO-mediated silencing of *Angptl3* in human subjects and mouse liver affects, in addition to circulating ANGPTL3, the intra-hepatocellular protein. In both humans and mice, similar results of ASO treatment were reported: In humans, the treatment resulted in a significant reduction of TG, LDL-C, VLDL-C, non-HDL-C, apoB-100 and ApoC-III [23]. In the siRNA-mediated Angptl3 knock-down study of Xu et al. [32], lipid phenotypes resembling the above were observed in both wild-type and obese mice, while in more ‘human-like’ *hCETP/ApoB-100* double transgenic animals, no drop of HDL-C was evident. Further, silencing of ANGPTL3 in HuH7 and HepG2 hepatocellular carcinoma cell lines caused a reduction in apoB-100 and TG secretion. Likewise, ANGPTL3 KD in non-cancerous

immortalized human hepatocytes (IHH) induced a shift from the secretion of large VLDL1-like particles to more lipid-poor VLDL2-like particles upon insulin stimulation [30]. Taken together, these observations suggest that intracellular ANGPTL3 acts as a regulator of VLDL secretion. However, the underlying mechanism(s) remain poorly understood. In a recent report, Ruhanen et al. [60] showed that the cholesterol ester (CE) levels are reduced in ANGPTL3 depleted hepatocytes, apparently mediated by downregulation of acyl-CoA:cholesterol acyltransferase1 (*SOAT1)* expression. Burnett et al. [63] have previously demonstrated a role of SOAT1 in VLDL secretion from hepatocytes, and the cellular CE levels are reported to affect apoB-100 and VLDL secretion [64,65]. The defects observed in VLDL secretion upon ANGPTL3 depletion may thus, in part, depend on the availability of intra-hepatocellular CE.

Another clue to the mechanisms that could putatively explain the impact of ANGPTL3 on VLDL secretion is provided by the study of Ruhanen et al. [60], who showed that ANGPTL3 knock-down hepatocytes are enriched with polyunsaturated fatty acid (PUFA)-containing lipid species (see paragraph 7). The changes in the FA composition may affect the assembly, composition and secretion of VLDL particles and also their further hydrolysis properties by LPL. Importantly, n-3 and n-6 PUFA intake in humans has been shown to reduce plasma VLDL, mainly by enhancing VLDL lipolysis and uptake [66]. On the other hand, lysophosphatidylcholine acyltransferase 3 (*LPCAT3*) deficient hepatocytes depleted of arachidonic acid (20:4n-6) containing phospholipid species were unable to secrete mature VLDL particles, apparently due to defects in forming the membrane curvature required for VLDL assembly [67]. This illustrates well how a change in the FA composition of lipids can modulate VLDL secretion. More studies, however, are required to determine whether the PUFA enrichment in ANGPTL3-depleted hepatocytes indeed affects the secretion of VLDL. We are tempted to speculate that the accumulation of PUFAs might favor the production of small VLDL particles or ones acting as a better substrate for LPL and hepatocellular uptake.

6. Connection of ANGPTL8 and ANGPTL3 function in the liver

Human ANGPTL8, a functional partner of ANGPTL3, is predominantly expressed in the liver, while mouse Angptl8 is expressed in both the liver and the adipose tissues. Studies on ANGPTL8 have provided evidence for intracellular functions in the regulation of metabolism and inflammatory responses. ANGPTL8 was reported to alleviate insulin resistance in

HepG2 cells via the Akt-GSK3β or Akt-FOXO1 pathway [68]. Moreover, ANGPTL8 oligomers were demonstrated dampen NF-κB activation through selective autophagic degradation of IKKγ in HepG2 cells, with consistent *in vivo* observations made in LPS-treated mice [69]. Importantly, ANGPTL3 relies on ANGPTL8 in the regulation of LPL-mediated TG hydrolysis: ANGPTL8 promotes the ability of ANGPTL3 to bind and inhibit LPL [70]. On the other hand, ANGPTL8 itself has a functional LPL-inhibitory motif, but inhibits LPL and increases plasma TG levels only in the presence of ANGPTL3 [71]. ANGPTL8 can form complexes with ANGPTL3 with varying stoichiometric ratios, the assembly of these complexes taking place intra-hepatocellularly. In this process ANGPTL8 binds to the N-terminal domain of ANGPTL3 and mediates proteolytic cleavage of ANGPTL3 via exposure of its cleavage site or recruitment of relevant hepatic proteases [7,72] (Fig. 3). Thus far there are no data available on a putative role of ANGPTL8 in hepatic lipid metabolism under conditions in which the liver is devoid of ANGPTL3 biosynthesis, i.e. FHBL2.

7. Impacts of ANGPTL3 on the fatty acid (FA) composition of hepatocyte lipids and on PUFA-derived lipid mediators

To date, only a handful of studies have addressed the putative effects of ANGPTL3 on intrahepatic lipid metabolism and in even fewer studies detailed information on intrahepatic lipid molecular species composition or FA profile has been reported. The limited information on how ANGPTL3 affects hepatic FA has been gained from animal or *in vitro* studies. In a study conducted using mice kept on a high-fat high-cholesterol diet, neutralizing ANGPTL3 in the circulation with a multiple dose treatment of the antibody Evinacumab (REGN1500) did not change the hepatic expression of genes related to *de novo* lipogenesis or FA oxidation, and, consistently, there was no change in liver fat content [19]. Similarly, there were no changes in the transcription of genes involved in fatty acid synthesis, desaturation, or β-oxidation in the livers of *Angplt3*$^{-/-}$ mice [33]. In these knock-out mice on a chow diet, the hepatic level of FA 18:2n-6 (linoleic acid) or 16:1n-7 (palmitoleic acid) did not change nor did the ratio of the exogenous FAs 18:2n-6 or 18:3n-3 (α-linolenic acid) to 16:1n-7.

In cultured human hepatocytes subjected to ANGPTL3 knock-down (KD), however, changes in lipid metabolism and in the FA composition of lipids have been observed. Both transcriptomics and lipidomics were employed to study the effects of *ANGPTL3* KD in IHH [60]. ANGPTL3 depletion led to statistically significant changes in several pathways related to lipid metabolism, such as the KEGG pathways ‘Fatty acid

metabolism', 'Biosynthesis of unsaturated fatty acids', 'Fatty acid elongation', 'Fatty acid degradation', 'Fatty acid biosynthesis', and 'Arachidonic acid metabolism'. These observations were accompanied by significant alterations in the total FA profile of the cells, showing a reduction of monounsaturated FA (MUFA) and an increase in both n-6 and n-3 PUFA. The relative MUFA depletion and PUFA increase were reflected in the lipid species profiles of major membrane phospholipids, the total levels of which, however, were not affected by the ANGPTL3 KD. In addition, there were similar FA changes observed in the species profile of CE, and the total CE level was reduced upon ANGPTL3 depletion. Interestingly, Xu et al. [32] found that in *ANGPTL3* KO human hepatocellular carcinoma cells, depletion of ANGPTL3 resulted in intracellular TG and cholesterol accumulation as well as an increase of long-chain TG, TG 58:8 being the single most affected TG species. Inversely, short-chain TG and diacylglycerols decreased in the ANGPTL3 depleted cells.

Both n-3 and n-6 PUFA are precursors of bioactive lipid mediators, important signaling molecules orchestrating inflammation and its resolution. The imbalance of pro-resolving and pro-inflammatory lipid mediators plays a role in the development of atherosclerotic CVD [73-77]. ANGPTL3 KD resulted in our IHH model in an enhanced production of lipid mediators [60], likely as a consequence of both increased substrate PUFA availability and upregulation of mRNA expression of cytosolic phospholipase A2, which releases PUFA from membrane phospholipids [78,79]. Most of the mediators elevated in the ANGPTL3 KD hepatocytes have known functions in promoting the resolution of inflammation, recovery from cardiovascular events, protection from ER stress, or attenuation of insulin resistance and hepatic fat accumulation [80-85]. However, since also the production of several 20:4n-6 derived pro-inflammatory lipid mediators was enhanced in the ANGPTL3 KD cells, one should interpret the results with caution. Concerning the main topic of this review, the changes observed in the FA composition of hepatocellular lipids as well as the alterations of PUFA-derived lipid mediators are no doubt due to cell-autonomous effects of ANGPTL3 KD. They can be mediated through altered expression or activity of lipid-modifying enzymatic machineries, and possibly the apparatus responsible for the uptake and metabolism of lipids, FA and glucose from the growth medium.

8. What does the plasma metabolome of ANGPTL3-deficient human subjects tell about their hepatic metabolism?

Human plasma metabolome analysis revealed that *ANGPTL3* LOF variant carriers have significantly higher concentrations of 3β-hydroxybutyrate (a ketone body) compared to normal human subjects, which may reflect enhanced hepatic β-oxidation of FA in the LOF variant carriers [86]. This could obviously result in reduced TG synthesis and packaging into newly synthesized VLDL particles, and thus be one of the mechanisms contributing to the low plasma apoB-100 lipoprotein levels in ANGPTL3-deficient or -depleted subjects. Of note, Wang et al. [31] found no evidence for enhanced hepatic FA oxidation in mice treated with Angptl3-inactivating antibody, consistent with the view that intra-hepatocellular KD of the gene may be required for such an effect. Similar to β-hydroxybutyrate, lactate was consistently higher at fasting and after a meal challenge in human subjects with complete *ANGPTL3* deficiency [86]. This may indicate an enhanced conversion of pyruvate to lactate instead of its routing to the acetate pathway, consistent with the reduced concentration of acetate observed in the LOF carriers. These observations suggest that ANGPTL3 deficiency causes a modest but significant shift in the hepatic energy substrate utilization.

9. ANGPTL3 and insulin sensitivity

ANGPTL3 LOF carriers exhibit enhanced insulin sensitivity and low plasma free FA levels [16]. The decrease in free FAs observed in these subjects could be the result of reduced adipocyte lipolysis in the absence of ANGPTL3 [62]. Moreover, there is evidence for a regulatory link between insulin and ANGPTL3: Plasma ANGPTL3 was reduced upon insulin infusion in human subjects, and insulin treatment suppressed the expression and secretion of ANGPTL3 by IHH [6]. A recent study utilizing non-human primates shows that the plasma concentration of ANGPTL3 was elevated in fructose induced insulin resistance and correlated positively with HOMA-IR, an index of insulin resistance [87]. In an elegant study on *Angptl3*$^{-/-}$ mice, Wang et al. [33] demonstrated an increase of insulin sensitivity, and reduced FA but clearly elevated glucose uptake into WAT in the fed state. This alteration in WAT substrate utilization could provide an explanation for the improvement of insulin sensitivity observed in both humans and mice deficient in ANGPTL3. A consistent effect of *Angplt3* inactivation on insulin sensitivity in mice treated with ASO was reported by Graham et al. [23]. Ruhanen et al. [60] showed that PUFA-containing lipid species were enriched in ANGPTL3 KD hepatocytes and that a similar PUFA enrichment was present in the TG of lipoproteins derived from human *ANGPTL3* LOF homozygote carriers. The impact of this

altered lipid composition of the lipoproteins in the ANGPTL3 deficient subjects remains an intriguing topic of future study. However, it might affect the quality of FAs stored in adipocytes but also in tissues involved in ectopic lipid storage, promoting insulin sensitivity. To conclude, the mechanisms through which depletion of ANGPTL3 improves whole body insulin sensitivity are thus far not well understood. However, present evidence suggests that reduced FA uptake into WAT and lipolysis, as well as elevated glucose uptake into WAT may play roles here. Further, the enrichment of circulating lipoproteins with PUFA in subjects with low ANGPTL3 activity could favorably modify the fatty acid composition of tissues crucial for insulin sensitivity. Moreover, ANGPTL3 KD was reported to reduce gluconeogenic gene expression and reduce the secretion of TG-rich VLDL by hepatocytes in culture [30], with putative insulin sensitizing effects.

10. Which physiological functions of ANGPTL3 can be considered cell autonomous?

A number of *in vitro* findings made by employing cultured hepatocyte models suggest cell-autonomous functions of ANGPTL3 in controlling hepatocellular VLDL secretion, β-lipoprotein uptake [32], or the insulin regulation of VLDL assembly/secretion [30]. Since functional impact in cultured hepatocytes cannot be attributed to alterations of LPL activity and the resulting FA fluxes, the above *in vitro* observations almost certainly reflect cell-autonomous functions of the protein. The same holds true for the changes observed in the FA composition of lipids, and the alterations in the transcriptome of cultured IHH subjected to ANGPTL3 KD [60]. We are tempted to speculate that the alterations in gene expression observed in the hepatocytes depleted of ANGPTL3 could relate to the altered FA metabolism, considering that FAs and their derivatives regulate a number of key nuclear receptors or transcription factors, such as peroxisome proliferator activated receptors (PPAR), hepatic nuclear factors (HNF), retinoid X receptors (RXR), and sterol regulatory element binding protein 1c (SREBP-1c) [88-91].

In vivo observations are more difficult to interpret from the point-of-view of cell autonomy, due to the complexity of biological systems. Most of the effects of ANGPTL3 antibody inhibition observed in mouse and man [19,21,22,24,31,42] can be attributed to the activation of LPL and resulting alterations in FA fluxes between lipoproteins and tissues, as well as the resulting indirect effects on hepatic lipoprotein production. However, the *in vivo* plus *in vitro* ANGPTL3 KD study of Xu et al. [32] contains truly interesting evidence for a role of ANGPTL3 in controlling hepatic lipid metabolism cell-autonomously, demonstrating an

enhancement of hepatic LDL/VLDL uptake, and provides evidence suggesting that this is due to elevated expression of LDLR and LRP1 by ANGPTL3-depleted hepatocytes (Fig. 3). Of note, significant reduction of LDL-C is observed not only in human subjects treated with ANGPTL3 ASO [23] but also upon Evinacumab treatment [21,22,24]. This suggests that an intrahepatocellular LDLR/LRP1 induction cannot be the major mechanism underlying the LDL-C lowering effect. It rather implies that altered fatty acid fluxes modulating hepatic VLDL assembly/secretion and the turn-over properties of the resulting β-lipoproteins must play a more important role.

One may wonder by what mechanism ANGPTL3, which has an N-terminal signal sequence, is synthesized through the endoplasmic reticulum (ER) membrane into the ER lumen and is secreted by hepatocytes through the canonical secretory route, could execute intracellular or cell-autonomous functions. Obviously, it could participate in lipoprotein assembly within the secretory pathway luminal compartments, in the ER, the Golgi apparatus and within secretory vesicles (Fig. 3). This type of intracellular function has in fact been demonstrated for another crucial regulator of lipoprotein metabolism, the phospholipid transfer protein (PLTP), a secreted plasma factor that also plays a major role in the assembly of apoB-containing lipoproteins in hepatocytes [92,93]. The intracellular function of PLTP most likely involves transfer of phospholipids to the nascent apoB lipoproteins within the secretory pathway. However, PLTP has also been found within the nucleus of neuronal cells, where it is suggested to execute as yet poorly understood functions [94]. Similar to PLTP, ANGPTL3 could regulate VLDL assembly and secretion through an activity within the secretory pathway of hepatocytes, where it is activated by furin cleavage [95]. Whether an isoform of ANGPTL3 could be found localized to the cytoplasmic or nuclear compartments is thus far not known. In the NCBI database there are no alternatively spliced variants of ANGPTL3 that would lack a signal sequence – however, this does not exclude their existence, or the signal sequence could under some circumstances be masked. Moreover, the protein secreted to the cell surface could be internalized through the endocytic pathway (Fig. 3) and execute activity within the endo-lysosomal compartments, recycle to the *trans*-Golgi, or possibly even exit the endomembrane system. These intriguing questions warrant detailed study in the future.

An interesting finding published almost 20 years ago showed that ANGPTL3 is able to bind integrin, a cell adhesion molecule [96]. The authors demonstrated in their cell-

based assays with recombinant proteins and direct binding experiments that ANGPTL3 bound to $\alpha_V\beta_3$-integrin and was thereby able to induce endothelial cell migration and adhesion. The Fibrinogen-like domain (FLD) of ANGPTL3 was sufficient for the integrin binding and induction of angiogenesis. The role of integrin $\alpha_V\beta_3$ as a receptor for ANGPTL3 has been afterwards verified in other locations such as in kidney podocytes [97,98]. Interestingly, ANGPTL3 was in podocytes shown to induce actin filament rearrangement, mediated by integrin $\alpha_V\beta_3$-dependent FAK and PI3K phosphorylation and Rac1 activation. Actin cytoskeleton could also be a target of ANGPTL3–integrin action in the liver. Furthermore, the PI3K–Akt pathway is a crucial part of the signaling that controls TG metabolism in hepatocytes. We therefore speculate that hepatic ANGPTL3 deficiency could suppress integrin $\alpha_V\beta_3$/PI3K/Akt signaling and thereby affect liver TG homeostasis. ANGPTL3 secreted by hepatocytes could engage $\alpha_V\beta_3$ on the surface of the same or near-by cells, resulting in cell-autonomous regulation of TG metabolism and VLDL secretion (Fig. 3). Moreover, ANGPTL3 may represent a local liver-specific angiogenic factor regulating vascular responses by binding to $\alpha_V\beta_3$-integrin expressed on the liver endothelium.

To conclude, accumulating evidence suggests that manipulating the expression of hepatic ANGPTL3 does have cell-autonomous impacts on hepatocellular gene expression and lipid metabolism. The topic has thus far been addressed only in a handful of studies and definitely deserves more thorough investigation.

11. Conclusions and future perspectives

ANGPTL3 is a highly promising new cardiometabolic therapy target. Nucleic acid-based therapeutic approaches that dampen the hepatic expression of this protein not only affect its lipase inhibitor activity in circulation but also its putative, emerging intra-hepatocellular or hepatocyte cell-autonomous activities. The evidence thus far suggests that suppression of *ANGPTL3* expression has quite significant effects on the hepatocyte transcriptome [60], glucose uptake, insulin sensitivity [23,30], LDL/VLDL uptake [32], VLDL assembly/secretion [23,32], the PUFA content of hepatocyte lipids, and the concentrations of PUFA-derived lipid mediators [60]. While most of these putatively cell-autonomous impacts of ANGPTL3 manipulation may be beneficial in nature and contribute to the cardioprotective capacity of

ANGPTL3 suppression, it is important to realize that among them there may also be ones with potential to be harmful in long term. Therefore, detailed study of the intra-hepatocellular functions of ANGPTL3 is definitely warranted.

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Conflicts of interest

None.

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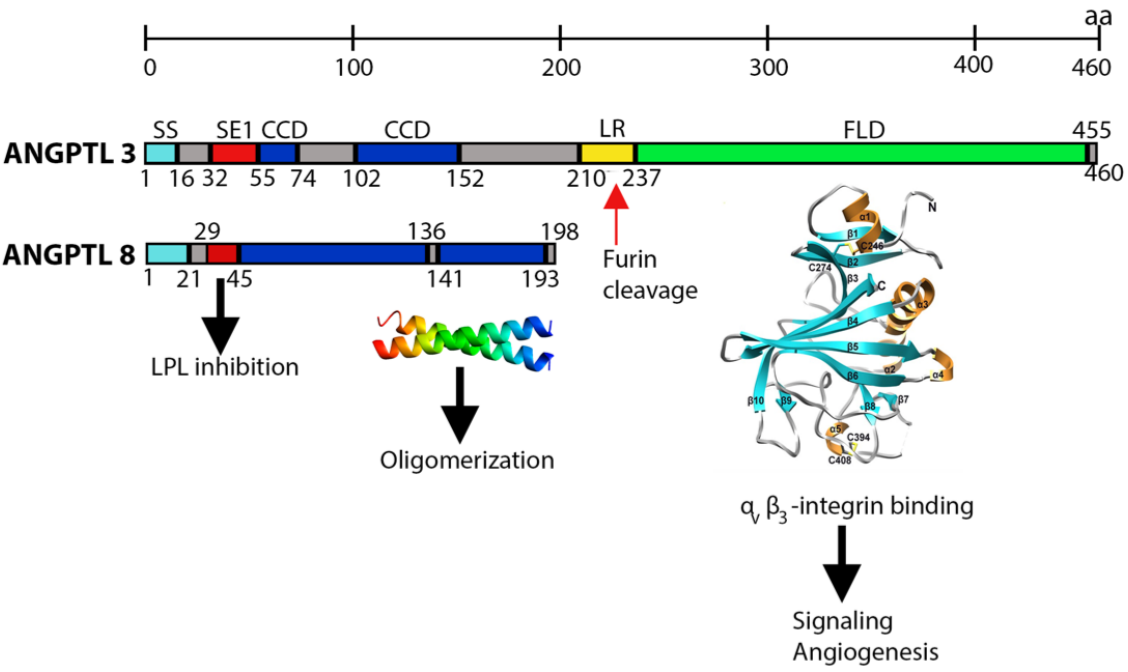
Figure captions

Fig. 1. The structure of ANGPTL3 and its functional partner ANGPTL8. Abbreviations: SS, signal sequence (translocation into the ER for secretion); SE1, LPL-specific epitope; CCD, coiled-coil domain; LR, linker region; FLD, fibrinogen-like domain; LPL, lipoprotein lipase. The SE1 epitope is required for binding LPL and inhibiting its activity. The coiled-coil domains mediate oligomerization of ANGPTL3 and -8 with each other and possibly with other partners. The FLD (absent in ANGPTL8) mediates binding of ANGPTL3 to its cellular receptor $\alpha_V\beta_3$-integrin, mediating signaling responses and the function of ANGPTL3 in angiogenesis. The image of ANGPTL3 FLD structure was reproduced from [99] under a Creative Commons license http://creativecommons.org/licenses/by/4.0/.

Fig. 2. The impacts of hepatic loss of *ANGPTL3* expression or its antibody-mediated inhibition in circulation, on metabolic fluxes in white adipose tissue (WAT), striated muscle and liver. The image collates data from studies of human subjects with loss-of-function variants, *Angptl3*$^{-/-}$ knock-out mice, pharmacologic inhibition studies in human or mice, and work carried out on cultured hepatocyte models. (A) Suppression of hepatocyte *ANGPTL3* expression reduces the post-prandial routing of fatty acids (FA) from triglyceride-rich lipoproteins (TRL) for storage in WAT, while increasing glucose (Glc) uptake into WAT and adipose *de novo* lipogenesis (DNL). Meanwhile it increases the hepatocyte uptake of Glc, LDL, and TRL remnants (TRL remn.), and inhibits the secretion of VLDL by the liver. (B) Inhibition of circulating ANGPTL3 with a humanized antibody (Ab) enhances the activity of LPL on TRL at oxidative tissues such as muscle, esulting in elevated FA uptake into these tissues and reduced FA flux to the liver. The reduced FA availability in the liver in turn reduces the hepatic secretion of VLDL triglycerides. Of note, the loss or knock-down of *ANGPTL3* in hepatocytes also results in the same metabolic effects as its inhibition in the circulation; The physiologic consequences of depleting *ANGPTL3* expression thus represent a combination of the effects in (A) and (B).

Fig. 3. The biosynthesis and putative cell autonomous functions of ANGPTL3 within hepatocytes. Points where ANGPTL3 can potentially execute cell autonomous functions are indicated with numerals (1-4). In the nucleus ANGPTL3 expression is regulated positively by liver X receptors (LXR) and hepatic nuclear factor 1α (HNF1α), and negatively by insulin, leptin, and thyroid hormone receptor β (TRβ). After synthesis and translocation into the endoplasmic reticulum, ANGPTL3 can form a complex with the related ANGPTL8, and the proteins are transported through the secretory pathway; Part of ANGPTL3 is on the way cleaved by the protease furin or other kexin-type proteases, which increases its biological activity. (1) Within the secretory pathway lumen ANGPTL3 may potentially regulate the assembly/lipidation/secretion of nascent VLDL particles. (2) After reaching the cell surface ANGPTL3 can via its fibrinogen-like domain bind to $\alpha_v\beta_3$-integrin or other as yet unknown receptors to induce signaling responses within the cell. These include the PI3K/Akt/mTOR pathway, which plays an important role in controlling VLDL assembly and secretion. Moreover, this pathway regulates glucose (Glc) uptake and metabolism. (3) ANGPTL3 bound to cell surface receptors could also be internalized via endocytosis, and execute intracellular functions within endosomes, or be routed from these organelles to the Golgi complex. (4) Depletion of ANGPTL3 in hepatocytes is reported to enhance the expression of LDL-receptor (LDLR) and LDLR-related protein 1 (LRP1), and to increase the hepatocellular uptake of LDL and TRL remnants (TRL remn). In addition to these four putative mechanisms, ANGPTL3 depletion is reported to increase the polyunsaturated fatty acid (PUFA) content of hepatocyte lipids, the synthesis of PUFA-derived lipid mediators, and possibly to stimulate the β-oxidation of fatty acids — processes which could reflect cell autonomous functions of the protein.

Fig. 1


aa
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100
200
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ANGPTL 3
SS
SE1
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102
152
210
237
460
ANGPTL 8
29
136
198
1
21
45
141
193
Furin
cleavage
LPL inhibition
Oligomerization
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C
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C246
C394
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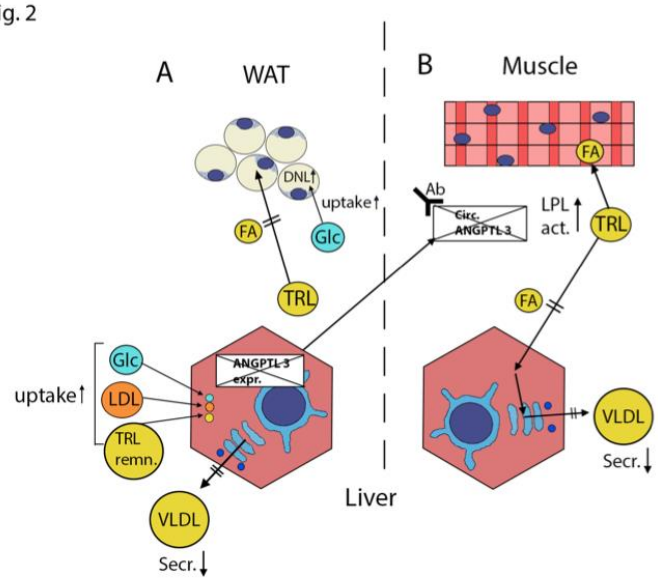
$\alpha_V\beta_3$-integrin binding
Signaling
Angiogenesis

Fig. 2


A
WAT
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Muscle
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Circ. ANGPTL-3
LPL act.↑
TRL
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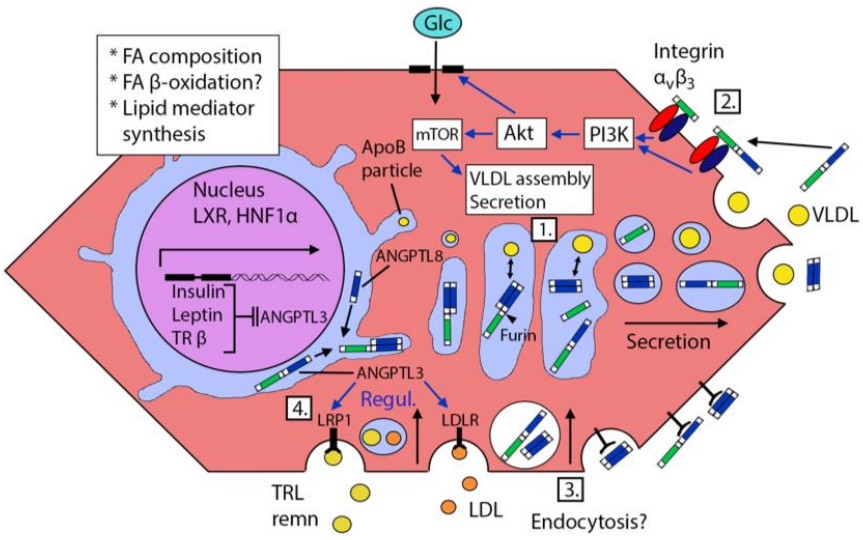
FA
TRL
Glc
LDL
TRL remn.
uptake↑
ANGPTL-3 expr.
VLDL
Secr.↓
Liver
VLDL
Secr.↓

Fig. 3


Glc
* FA composition
* FA β-oxidation?
* Lipid mediator synthesis
Integrin
$\alpha_v\beta_3$
2.
mTOR
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PI3K
ApoB particle
VLDL assembly Secretion
VLDL
Nucleus
LXR, HNF1α
1.
ANGPTL8
Insulin
Leptin
TR β
ANGPTL3
Furin
Secretion
ANGPTL3
Regul.
4.
LRP1
LDLR
TRL remn
LDL
3.
Endocytosis?